THE COUNCIL FOR TOBACCO RESEARCH–U.S.A., INC.
110 EAST 59TH STREET
NEW YORK, N.Y. 10022
(212) 421-8885

Application for Research Grant
(Use extra pages as needed)

Date: Jan. 24, 1975

Continuation Grant

1. Principal Investigator (give title and degrees):

Charles G. Cochrane, M. D. - Member

2. Institution & address:

Scripps Clinic and Research Foundation
476 Prospect Street
La Jolla, California 92037

3. Department(s) where research will be done or collaboration provided:

Department of Immunopathology

4. Short title of study:

The Mediation of Inflammatory Injury of Tissue

5. Proposed starting date: July 1, 1975

6. Estimated time to complete: July 1, 1976

7. Brief description of specific research aims:

1. To study the participation of the kinin forming, intrinsic clotting and fibrinolytic systems in inflammatory tissue injury.
   a. Purification of component proteins
   b. Analysis of mechanisms of activation in vitro
   c. Analysis of their participation in pulmonary disease
2. To study the participation of cellular factors in inflammatory injury

8. Brief statement of working hypothesis:

Inflammatory injury, induced by a wide variety of stimuli, is effected by a series of humoral and cellular mediators. While in many instances the causative inciting agents remain unknown, the mediation systems of the injury are common to them all and subject to analysis. Successful therapeutic resolution of inflammatory injury will only come about through careful analysis.

9. Details of experimental design and procedures (append extra pages as necessary)

I. Summary of proposals from original application.

A. The participation of the Hageman factor pathways in the pathogenesis of inflammatory tissue injury.

One of the most important and yet unknown areas in the pathogenesis of inflammatory disease lies in the kinin forming, intrinsic clotting clotting and fibrinolytic systems, otherwise known as the Hageman factor pathways. These systems are outlined in Table I

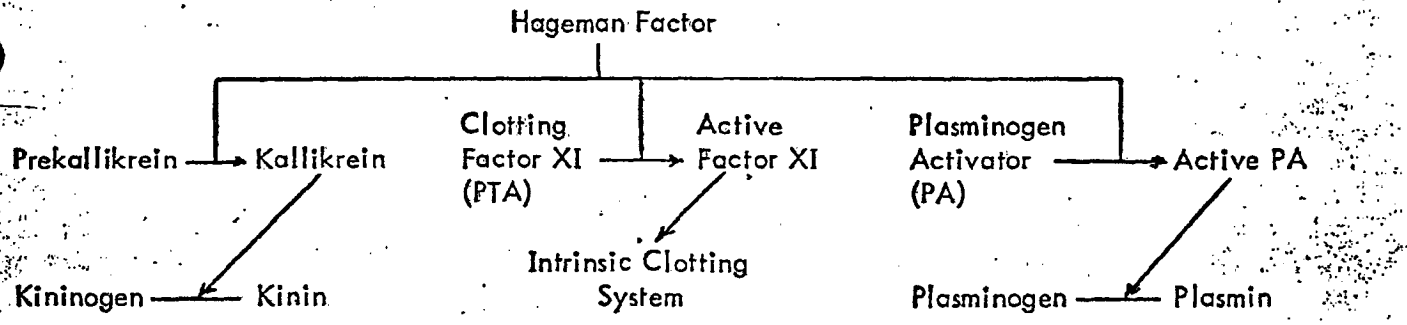

Table I

Activation of these systems otherwise known as the Hageman factor pathways leads to the development of many aspects of the inflammatory process:

1. The injection of activated Hageman factor intradermally induces increased vascular permeability and an influx of inflammatory cells.

2. Kinins cause an increase in vascular permeability and hypotension.

3. Fibrin formation can lead to permanent loss of function and may well stimulate formation of collagen and fibrosis (preventing fibrin deposition in glomerulonephritis blocks fibrosis of the glomeruli).

4. Fibrin and fibrinogen degradation products, generated by the action of plasmin, increase vascular permeability and are chemotactic to neutrophils.

5. Plasmin activates C1s leading to stimulation of the complement system.

6. Thrombin, a product of the intrinsic clotting system, can generate activity of C3.

We have devoted five years to the elucidation of these systems by isolating and characterizing components of each in precursor form, defining their mechanisms of interaction and developing methods by which their participation in inflammatory diseases can be evaluated. These studies, which have been supported by the Council for Tobacco Research, have been performed with both rabbit and human plasma. We have chosen rabbits since this species serves well for experimental studies in vivo. With human components, studies can be conducted in clinical disease states.

To date we have been able to isolate in purified form Hageman factor (HF), prekallikrein, factor XI, and plasminogen, from each species. Plasminogen activator has been prepared in impure form. Each protein has been radiolabelled successfully and antibodies prepared to almost all. The activation of HF has received considerable attention and two mechanisms have been defined: solid-phase activation, i.e., that occurring when HF interacts with a negatively charged surface, and fluid-phase activation, i.e., that occurring when enzymes in the plasma, notably kallikrein, act upon fresh HF. These studies have formed the basis of several publications (1,2,3,4). Further data are given in the section on Progress Report.

Continuing experiments, given in detail in the original report, are designed to examine the means by which the HF pathways, i.e., the kinin-forming, intrinsic clotting, and fibrinolytic systems participate in inflammatory tissue injury. In brief, these studies will be conducted as follows:

1. Studies of the participation of the HF pathway components in inflammatory pulmonary disease (see below) by:

   a) Determination of turnover times of ^{125}I labelled components
   b) Changes in levels of components in plasma during the development of injury
   c) Detection of activated components in plasma

2. Detection of activated components bound at the site of injury using fluorescent antibodies and ^{125}I labelled components.

3. Assessment of the effect of deprivation or specific inhibition of activated components on the development of experimental inflammatory lesions.

Several means instituting inflammatory injury of the lung will be employed:

1) Antibodies directed to pulmonary membrane infused intravenously; 2) repeated inhalation of vaporized soluble antigen into rabbits previously immunized; 3) inhalation of fine particulate kaolin and antigen bound to kaolin in a previously immunized rabbit; 4) repeated intravenous administration of soluble antigen-antibody complexes and bacterial lipopolysaccharide. In each type of injury, acute inflammation results, with influx of inflammatory cells and formation of fibrin. Repeated administration of the inciting agents leads to chronic inflammatory changes.

In addition, activation of various components of the HF pathways, e.g., HF, prekallikrein, factor XI, plasminogen by cellular enzymes will be sought. In particular, alveolar macrophages from normal rabbits and rabbits with pulmonary inflammation will be tested. Aside from assaying the enzymatic activity of the components, critical cleavage of the protein structure will be examined. Previous studies in this laboratory have indicated that ^{125}I-labelled precursor components are activated by limited proteolysis.

Finally, as written in detail in the previous year's renewal, the crystals found in alveolar macrophages of smoker's lungs will be obtained and tested for their ability to activate HF. These fine crystals have the chemical composition and physical appearance of kaolin, an excellent activator of HF. The crystals are released periodically from the macrophages at which time serious inflammatory injury could take place if HF were bound and activated. These pilot studies, if positive, would lead to a series of studies aimed at finding the importance of such an activation mechanism inflammatory injury of the lung.

B. Cellular mechanisms involved in inflammatory tissue injury

These studies are designed to examine the mechanisms of activation of inflammatory cells and of release of their injurious constituents. Four cell types have been chosen for study; platelets, mast cells, macrophages and neutrophils. A series of activators for each cell type have been selected: for platelets - collagen, $F(Ab)_2$ anti-platelet antibody, platelet-activating factor derived from basophils (13) or pulmonary mast cells (14) and thrombin; for mast cells - polymyxin B, 48/80, C3a and C5a anaphylatoxins and a newly described cobra venom protein that stimulates mast cells to release granular constituents (11); for macrophages - immune complexes, components of the HF pathways, insolubilized aggregated immunoglobulins of specific classes and subclasses; for neutrophils-insolubilized aggregated immunoglobulins of specific classes and subclasses and C3a and C5a anaphylatoxins.

The studies will include examination of binding of the activators to the cell surfaces, the numbers of molecules bound that are required for release of constituents to occur and a definition of the receptors on the outer membranes of the cells with which the activators react.

Studies conducted in this laboratory have already demonstrated that several of these activators in insoluble form are capable of activating fully the respective cells to release their granular or lysosomal constituents (9). Different receptors apparently exist on mast cells for different stimuli and on neutrophils and platelets for different classes of immunoglobulins (8,11,12).

The mechanisms by which these activators stimulate release will also be examined. The suggestion that different esterase enzymes are activated by each stimulating agent will be tested experimentally (see below).

These experimental plans are presented only briefly as they represent a condensation of material given in detail in the initial application.

Experimental Plans for the Coming Year

A. Participation of the Hageman factor activated pathways in the pathogenesis of inflammatory tissue injury

1. The consumption of circulating ^{125}I prekallikrein, plasminogen, fibrinogen and clotting factor XI will be determined in rabbits given 200 µg purified lipopolysaccharide (LPS) of E. coli 0111:B4 and of the mutant Re 595 LPS. The latter possesses only the 2-keto-3 octanoic acid linkage group and lipid A and is an avid activator of the alternative pathway of complement. The former LPS fails to activate complement detectably. Both are potent activators of HF. Both forms of LPS when injected intravenously induce an inflammatory reaction with deposition of fibrin in the lung. The specific consumption of the above components will be assessed by comparing rates of loss from the circulation of the particular component in comparison to the loss of rabbit albumin.

Uptake of ^{125}I labelled components in the lung and other organs will be determined. Histologic and immunofluorescent studies will reveal the type of inflammation and the location of components of the HF and complement pathways in relation to the presence of inflammatory foci. Particular attention will be paid to the presence of neutrophils, platelets and macrophages. Animals used in these immunopathologic studies will be sacrificied at intervals of 30 min, 1, 4, 8 and 18 hours after a single injection of LPS. Damage to pulmonary alveolar walls will be assessed by the rate of passage of ^{131}I rabbit albumin into alveolar fluid obtained then by lavage in rabbits sacrificed sequentially. Lavage fluid will also be used to test for ^{125}I fibrinogen split products.

Similar analyses will then be conducted in rabbits using as inciting agents: (1) antibodies from sheep directed to rabbit pulmonary basement membranes; and (2) aerosolized finely particulate kaolin (an excellent activator of the HF pathways) coated with bovine albumin in rabbits previously sensitized with the antigen.

In studies to be performed in the following year we will assess the affect of multiple administrations of these agents. Chronic changes, especially the development of fibrosis will be sought.

2. Depriviation studies to demonstrate the role of various factors in the development of inflammation.

In the currently proposed studies we will then deplete rabbits of cellular and humoral components to assess the effect on the development of inflammation and on the consumption and localization of other components as outlined above. The following studies will be performed initially:

a. Deprivation of neutrophils (by nitrogen mustard and specific anti-neutrophil antibody) and platelets (specific anti-platelet antibody).

b. Depletion of fibrinogen with commercially available snake venom enzyme (a technique we currently employ).

c. Blockade of the intrinsic clotting system by removing factor XI and factor VIII (trace proteins) with specific antibody. This method has proved successful in this and other laboratories.

d. Blockade of the extrinsic clotting system with antibodies to clotting factor VII. We are currently testing whether antibodies to bovine factor VII cross react sufficiently with rabbit factor VII to deplete this trace protein from the circulation. If the method does not succeed, we will purify rabbit factor VII to produce antibodies.

e. Removal of the third component of complement (C3) with cobra venom factor.

These deprivation studies are aimed at defining not only which components are important in the development of injury, but in what order they interact. A sequential activation sequence may then be recognized in the development of critical pulmonary injury.

3. The activation of components of the HF pathways by cellular constituents

Alveolar macrophages will be isolated from lungs of rabbits by lavage (Brain et al. Arch. of Int. Med. (26:477, 1970), a technique already employed in this laboratory, and the granules obtained by disruption of the cells in hypertonic sucrose. After lysis of the granules, the constituents will be assayed for their capacity to activate Hageman factor over a range of conditions of pH, temperature and ionic strength. Since Hageman factor, activated by enzymes such as plasmin or kallikrein is cleaved in the process, a similar effect of the granular constituents of alveolar macrophages will be examined. This will define the mechanism of activation as fluid phase rather than solid phase (i.e., when Hageman factor binds to negatively charged surfaces such as that provided by alveolar membranes). The implication would be that clotting of plasma, and release of kinins and plasmin would occur in the fluid of the alveolar spaces, thereby provoking a diffuse reaction.

The granular constituents of the alveolar macrophages will then be fractionated on DEAE Sephadex A50 under conditions of constant pH and varying salt concentration. Enzymes or other activators of Hageman factor will be measured and isolated and identified through additional chromatographic and physical methods.

B. Mechanism of activation of cells important in the development of inflammatory injury.

We have observed that neutrophils, platelets, and mast cells release injurious products following stimulation at the membrane surface. In addition, considerable specificity exists in what might be considered as a membrane "receptor". We will now begin experiments in which the membrane receptors will be isolated and characterized. The work will initially be performed on neutrophils since large numbers of the cells (10^{10}) can readily be obtained in pure populations and since those cells play a fundamental role in pulmonary tissue injury.

Membrane fragments will be obtained from isolated washed neutrophils by shear-breakage in 0.34 M sucrose followed by sucrose density gradient centrifugation. Plasma membranes are found (40-60% recovery) at the interface between 20 and 40% sucrose. Granule breakage is minimal. We will use 5' nucleotidase and ^{125}I-labelled antineutrophil antibody (incubated with an aliquot of intact neutrophils beforehand) to follow the membranes.

The membrane fragments will be radiolabelled with iodine (lactoperoxidase-H_2O_2 method) and examined for binding to EAC 3_b (C 3b receptor) or Sepharose aggregated γ globulin (γG receptor). Preliminary experiments indicate the feasibility of the approach and the ability to obtain fragments retaining the C3b receptor. A binding inhibition assay will be established using unlabelled membrane to inhibit the binding of ^{125}I membrane to EAC or Sepharose γG. This will allow quantitative assessment of recovery of receptor from solubilized membranes. Four methods of solubilization will be examined: a) detergents, e.g., NP40 and Triton X100. The latter appears useful for the C3b receptor and may be removed afterwards by the method of Holloway (Holloway, P.W., Analytical Chem., 53, 309, 1973). b) Ultrasound. Low frequency ultrasonication of the fragments (to avoid oxidative effects) will be assessed. c) Proteolytic enzymes. Trypsin removes the C3b receptor from intact neutrophils. While trypsin probably fragments the receptor protein, the binding portion of this molecule may still be obtained and indeed preliminary evidence suggests that receptor activity can be recovered. d) Chaotropic agents and KCl. The different agents and techniques will be applied to the membrane fragment. The solubilization will be assessed by sucrose density gradient ultracentrifugation, examining protein and radiolabel and the receptor will be followed by binding inhibition.

Following solubilization, the receptors will be isolated by several means:

1. Affinity chromatography (see below in the mast cell section) using Sepharose-γG or zymosan particles with C3b attached (ZC) will entail binding of receptors to such particles followed by elution of the receptor molecules. For example, rabbit C3b receptors require Mg++ (Henson, Immunology, 1969) and thus may be eluted with EDTA.

2. Standard chromatographic procedures (with which this laboratory has much experience) will also be used to isolate the receptor molecules. These methods will include

gel exclusion, ion exchange and hydroxyappatite chromatography, polyacrylamide gel electrophoresis, isoelectric focusing and pevikon electrophoresis. The receptors will thus be isolated and simultaneously characterized as to molecular weight, charge and composition.

3. The C3b receptor is presumably a protein (from its trypsin sensitivity) and is possibly a glycoprotein. Thus another approach to its isolation is to determine whether lectins e.g., concanavalin A or phytohemagglutinin, will bind to the receptor protein. If C3b binds to Sepharose-Con A, then this receptor may be eluted from the Sepharose-lectin with a-methyl mannoside.

Once isolated, the receptor will be labelled (e.g., with iodine if it is a protein) and studied for its binding properties and biologic activity. To indicate that it indeed may be the molecule involved in the cell activation, activation inhibition experiments, where the receptor is used to inhibit ZC induced phagocytosis on enzyme release, will be performed. Antibody to the receptor will be prepared and Fab fragments isolated. This antibody will then also be used to block neutrophil activation. The binding of radiolabelled receptor to EAC_{3b} or ZC_{3b} will be studied. What is the ratio of receptor to C3b in the erythrocyte? What is the nature of the bond, i.e., is it inhibited by salt or by detergents, indicating ionic or hydrophobic interactions (see section 1).

A question of considerable interest and importance which may then be answerable is whether C3b and C3a, both activators of neutrophils, may bind to and activate the same receptor.

C3b receptors will be studied for their ability to bind and remove active C3a. C3a will be assayed by anaphylatoxin activity or by neutrophil chemotaxis. These studies will allow us to follow C3b binding molecules during purification.

12. Biographical sketches of investigator:

Charles G. Cochrane:

REDACTED

Education: University of Rochester, B.A.; M.D., School of Medicine

Professional Record: Department of Microbiological Chemistry (Prof. P. Grabar), Institut Pasteur, Paris, 1959-60; Assistant Professor, University of Pittsburgh, School of Medicine, 1960-61; Associate, Division of Experimental Pathology, Scripps Clinic and Research Foundation, 1961-64; Associate Member, 1964-67; Member, 1967-present; Professor of Pathology in Residence, University of California School of Medicine, 1968-present

Fellowships and Honors: Student Fellowship, Department of Pathology, University of Rochester, 1953-54; Sarah Mellon Scaife Fellow, Department of Pathology, University of Pittsburgh, School of Medicine, 1956-60; Helen Hay Whitney Research Fellow, 1961-64; Established Investigator, The Helen Hay Whitney Foundation, 1964-69; John M. Sheldon Memorial Lectureship of the American Academy of Allergy, 1968; Parke, Davis Meritorious Award in Experimental Pathology for the Year, 1969.

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Consultantships: Member, Pathology Training Committee, National Institute of General Medical Sciences, USPHS; Fellowship Committee, The Arthritis Foundation; Member of the Advisory Committee, National Multiple Sclerosis Society.

Editorial Boards: The Journal of Clinical and Experimental Immunology, The American Journal of Pathology, Associate Editor, The Journal of Immunology.

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12. Biographical sketeches of investigators:

Peter M. Henson:

REDACTED

Education: High School in Britain and Rhodesia

Bachelor of Veterinary Medicine and Surgery (BVM&S), Edinburgh University, Edinburgh, Scotland, R William Dick Medal for best graduate.

First Class Honors, B. Sc. in Bacteriology, Edinburgh University, Edinburgh, Scotland, R

Ph. D., University of Cambridge, Cambridge, England, R Thesis: "The Biological Activity of the Third Component of Complement".

Professional Record:

Research Fellow, Department of Experimental Pathology, Scripps Clinic and Research Foundation, La Jolla, CA 92037

Associate, Department of Experimental Pathology, Scripps Clinic and Research Foundation, La Jolla, CA 92037

Associate Member, Department of Immunopathology (same as above), Scripps Clinic and Research Foundation, La Jolla, CA 92037

Memberships:

REDACTED

REDACTED

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12. Biographical sketches of investigators:

Stephen W. Russell

REDACTED

Education:

University of California, Davis, REDACTED B. S.

University of California, Davis, REDACTED D.V.M.

Internship, Angell Memorial Animal Hospital, Boston, Massachusetts, REDACTED

University of California, Davis, REDACTED and University of California, San Francisco, REDACTED Ph. D.

Professional Record:

Postdoctoral Research Fellow, Scripps Clinic and Research Foundation, La Jolla, CA 1972-1973

Associate, Scripps Clinic and Research Foundation, La Jolla, CA 1973-present

Awards and Academic Honors:

Special Fellow, United States Publich Health Service, 1970 & 1971
Outstanding Clinical Research Project, Angell Memorial Animal Hospital, 1967
University of California School Medalist, 1966
Graduated 1st in Veterinary Medical School Class, 1966
Outstanding Clinician, Veterinary Medical School Class, 1966
Hart Memorial Scholarships, 1964 and 1965
B. S. Degree with Honors

Professional Organizations:

REDACTED

REDACTED REDACTED

12. Biographical sketches of investigators:

Richard J. Ulevitch, Ph. D.

REDACTED

Education:

A.B., Liberal Arts, Washington and Jefferson College, R

Ph. D., Biochemistry, University of Pennsylvania, R

Professional Record:

1971 - 1972, Postdoctoral fellow, University of Minnesota with Drs. Beulah Holmes Gray and Robert A. Good

1972-1974, Research Fellow, Scripps Clinic and Research Foundation, Department of Immunopathology, La Jolla, CA 92037

1974-present, Research Associate, Department of Immunopathology, Scripps Clinic and Research Foundation

Progress Report

1. Participation of the intrinsic clotting, kinin forming and fibrinolytic systems in disease

a. Biologic activities of the first component, Hageman factor (HF)

Purified, radiolabelled HF has been examined as to its physical properties and biologic activities. These studies are of significance in that when HF is activated, it binds (solid phase activation) to the negatively charged activator, leading to generation of numerous potentially injurious agents: kinins, fibrinogen split products, fibrin; activated C1s, neutrophilic chemotactic factors and active esteratic enzymes.

The amino acid composition of HF was determined (1). This will be of help in future studies of the residues important in the biologic activities of HF. Antibodies were produced to human HF and quantitative determinations of the molecule in plasma or other fluids is possible for the first time. Normal values range from 14 to 47 µg/ml plasma with an average value of 29 µg/ml. Studies on the levels of this important protein in disease are underway.

As noted in last year's report, HF is cleaved by enzymes that it activates which, in a reciprocal fashion, activate additional HF. We have termed this "fluid-phase" or enzymatic activation (2,3, 4). The HF molecule is cleaved into fragments of 52,000, 40,000 and 28,000 daltons. The 28,000 MW fragment contains the enzyme sites of the molecule (1). We have recently observed that the sites responsible for binding the HF to negatively charged surfaces (solid-phase activation) lie in the 52,000 and 40,000 MW fragments. When HF, bound to and activated by a negatively charged surface (such as provided by collagen or extracellular basement membranes of the lung) is exposed to its substrates in plasma, namely prekallikrein and plasminogen. These enzymes cleave fresh, native HF and activate it. They also cleave the bound HF, and release the 28,000 enzymatically active fragment into the supernatant (2, 4). The active fragment then becomes rapidly bound to an inhibitor which is most probably C1 inhibitor: the fragment does not bind to the inhibitor in C1 inh. deficient plasma; the fragment binds to purified C1 inh. and the 28,000 MW fragment changes its electrophoretic mobility in whole plasma to become superimposable with the C1 inh.

From these studies, one may anticipate that HF when activated in diseases will be found along membranes where it is bound. The portion bound will be the 52,000 and 40,000 M.W. fragments only, while the 28,000 M.W. fragment will be present in the plasma, bound to C1 inhibitor. We are currently designing assays for the activated 28,000 M.W. fragment in plasma. It is our feeling that far more sensitive assays can be obtained for the activated HF than for the residual native molecules. The same theory applies to kallikrein, factor XI and plasmin.

b. Purification of rabbit plasminogen, fibrinogen, clotting factor XI and prekallikrein for assays of consumption during inflammatory injury.

In order to examine the participation of these proteins in inflammatory pulmonary injury, it is essential to obtain them in purified, precursor form. Radiolabels can then be applied and antibodies obtained for detection in plasma, alveolar fluid and tissues. Accordingly, we have obtained each of these in precursor form by dermatographic methods. While inactive as they exist, each can be converted to its active form by treatment with its natural activator, i.e., plasminogen activator, thrombin, and HF respectively. We are now obtaining turn over times of the radiolabelled molecules in normal rabbits prior to performing studies of turn over (consumption) and tissue-localization in inflammatory conditions of the lung (see original report). Antibodies are now prepared to fibrinogen and plasminogen for quantitative assays, and goats are being immunized with prekallikrein and factor XI.

c. Action of alveolar macrophages on components of the HF pathways.

The initial studies of the activation of prekallikrein and HF by alveolar macrophages have been made difficult by the low concentrations of the components in solution. We have therefore expended considerable effort in developing a new method for preparing components of the HF pathway that will yield much greater quantities. The method, as reviewed in the section on experimental protocols for the ensuing year, involves a computer programmed method of preparatory polyacrylamide electrophoresis (Chrombach and Rodbard). We have established the computer programs which describe approximately 5,000 buffer conditions available for isolating any given protein by its physical properties. We have applied the technique to rabbit prekallikrein with extraordinary results: far greater amounts of the prekallikrein were obtained than before in purer form. When the proteins of the HF pathway are prepared in adequate concentration by the new method, we will continue the studies that appear most promising: the activation of components of the HF pathways by enzymes obtained from alveolar macrophages.

d. Activation of Hageman factor by bacterial lipopolysaccharide.

Hageman factor was formed to be activated by purified bacterial lipopolysaccharide (LPS) (5,6). The lipid A moiety was determined to be the active portion in the LPS structure. Hageman factor bound to the LPS and was active in the complexed form. Cleavage of the molecule did not occur.

e. The identification of PF/dil.

When plasma is diluted with saline in the presence of a negatively charged surface, a permeability factor (PF/dil) is generated. The nature of this substance has remained unknown since its discovery in 1953, although its interaction with the Hageman factor pathway appeared certain. Using insolubilized antibodies to Hageman factor and other components of the pathway, we determined that PF/dil was identical with activated Hageman factor (7,15).

2. Mechanism of activation of neutrophils, platelets and mast cells for release of injurious constituents.

Studies were continued into the mechanisms of activation of inflammatory cells and release of their injurious constituents. These studies were designed to mimic conditions occuring in inflammatory foci where neutrophils, platelets, macrophages, mast cells and lymphocytes release constituents that injure surrounding tissues. The stimulation of platelet surfaces was brought about by several agents: $F(Ab)_2$ anti-platelet antibody, platelet activating factor (PAF) derived from basophils and pulmonary macrophages, collagen and thrombin. Each agent induced release of stored 3H serotonin (8). To determine whether these chemically disparate agents activated release mechanisms in the platelet by different means, the characteristics of the esterase enzymes which are activated early in the release process were examined. Using phosphonate inhibitors, synthetic ester substrates, and plant-derived inhibitors of trypsin, it was found that each activator activated esterases in the platelets possessing different inhibitory spectra. This strongly suggested different esterases are involved and that specific receptors are activated by each of the above stimuli.

With mast cells derived from peritoneal cavities of rats, two types of activators were described that induce release of histamine and serotonin. Type I activators included compound 40/80 and polymyxin B while Type II activators included C3a anaphylatoxin, a cobra venom protein recently described in this laboratory, and band 2 protein of neutrophils (9, 16). These activators were found to stimulate the mast cells at their surface (10), for insolubilization of the activators on Sepharose 4B allowed full activation to occur. The differentiation into two types of activators was determined by blocking the binding of one type of activator (radiolabelled with ^{125}I) with members of that type, but not the other, and by the ability of activators of one type to desensitize mast cells to activators of that type, but not the other (11, 12).

THOMAS CLARKE KRAVIS, M. D.

REDACTED

Education:

Fairfield University
Fairfield, Connecticut
B.S. Biology - cum laude - R
Activities and honors: Dean's list

Jefferson Medical College
Philadelphia, Pennsylvania
M. D. - R
Activities and honors: Dean's committee; student council; vice president; director: Project Haiti; president: Student AMA

University of California, San Diego
Straight medical internship - Dr. E. Braunwald, 1968-1969
Medical resident - Dr. E. Braunwald, 1969-1970
Pulmonary clinical fellow - Dr. K. Moser, 1970-1971
Pulmonary research fellow - Dr. N. Zvaifler, 1971-1972
Research fellow - Dr. C. Cochrane - Scripps Clinic & Research Foundation, 1972-
Clinical instructor of medicine - U. C. S. D., 1972-

Activities:

Director medical clinic teaching program - Tijuana Mexico (1969); Instructor student medical clinic (1971); Coordinator pulmonary rehabilitation course (1971); lectures in inhalation therapy, Grossmon College (1970); Director alpha-1-anti-trypsin laboratory, U. C. S. D. and coordinator alpha-1-antitrypsin SCOR study.

Current studies:

The role of IgE and the basophil in immune complex disease; platelet activating factor in asthma; leukocyte dependent histamine release in man and rabbit. Antigen-antibody crossed gel electrophoresis for alpha-1-antitrypsin phenotypes.

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10. Space and facilities available (when elsewhere than item 2 indicates, state location):

Approximately 2,000 sq. ft. of laboratory space is available. The laboratory is equipped with refrigerated centrifuges, electrophoretic and chromatographic equipment, five fraction collectors, a walk-in cold room, deep freezes (-20° and -70°), complete fluorescent equipment and a preparative ultracentrifuge. Histologic and electron microscopic analyses are carried out in facilities available and frequently used by the applicants. An analytical ultracentrifuge and amino acid analyzer are also used by the applicants within the department. Complete isotope labeling, hot lab and monitoring facilities are in current use. Two Schultz-Dale bath apparatuses are used for assays of biologically active materials.

11. Additional facilities required:

None

12. Biographical sketches of investigator(s) and other professional personnel (append):

Charles G. Cochrane, M.D.; Peter M. Henson, Ph.D.; Stephen W. Russell, DVM, Ph.D.; Richard J. Ulevitch, Ph.D.; Thomas C. Kravis, M.D.

13. Publications: (five most recent and pertinent of investigator(s); append list, and provide reprints if available).

4.

14. First year budget:

A. Salaries (give names or state "to be recruited") Professional (give % time of investigator(s) even if no salary requested)	% time	Amount
Charles G. Cochrane	20	
Peter M. Henson	20	
Stephen W. Russell	50	
Richard J. Ulevitch	50	
Fringe Benefits – S. W. Russell R. J. Ulevitch		
Thomas C. Kravis Technical	50	
Part time services of animal caretaker and dishwasher		
Fringe Benefits		
	Sub-Total for A	
B. Consumable supplies (by major categories)		
Chemicals, proteins		1,500
Glassware, plasticware		1,350
Animals, feed and bedding		1,000
	Sub-Total for B	3,850
C. Other expenses (itemize)		
none		---
	Sub-Total for C	---
	Running Total of A + B + C	22,450
D. Permanent equipment (itemize)		
none		
	Sub-Total for D	
E. Indirect costs (15% of A+B+C)	E	3,368
	Total request	25,818

[redacted]

15. Estimated future requirements:

	Salaries	Consumable Suppl.	Other Expenses	Permanent Equip.	Indirect Costs	Total
Year 2						
Year 3						

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16. Other sources of financial support:
List financial support from all sources, including own institution, for this and related research projects.

CURRENTLY ACTIVE

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Immunologic Studies	United States Public Health Service AI07007	$150,000	1975-1976
The Mediation of Inflammatory Injury of Tissues	Council for Tobaccco Res.	23,400	1975-1976

PENDING OR PLANNED

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Principal investigator

Typed Name Charles G. Cochrane, M. D.

Signature [signature] Date Jan. 28, 1975

Telephone 714 (Area Code) 459-2390 (Number) 306 (Extension)

Checks payable to

Robert Erra

Mailing address for checks

Scripps Clinic and Research Foundation

476 Prospect Street, La Jolla, CA 92037

Responsible officer of institution

Typed Name Jacinto J. Vazquez, M. D.

Title Assistant Director

Signature [signature] Date Jan. 28, 1975

Telephone 714 (Area Code) 459-2390 (Number) 223 (Extension)

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References

1. Revak, S., Cochrane, C., Johnston, A., and Hugli,T.: Structural changes accompanying enzymatic activation of human Hageman factor, J. Clin. Invest., 54: 1974.

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